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MEMBRANE POTENTIALS IN ISOLATED NEURONES *IN VITRO* FROM DEITERS' NUCLEUS OF RABBIT

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The biochemical properties of isolated neurones of Deiters' nucleus of rabbit have been the subject of considerable study (for review, see Hydén, 1961), but their physiological status has not been investigated. In the present study, the ability of the isolated neurones of Deiters' nucleus to maintain resting potentials at 23° C has been demonstrated, and the effect of hypoxia, different medium glucose, potassium and sodium concentrations, and a ganglioside preparation has been examined. The latter agent was tested as it had been found to increase the membrane potential of cells in guinea-pig cerebral slices *in vitro* (Hillman, 1961).

METHODS

Apparatus

A chamber was designed which would fit on a microscope stage, and allow the micro-electrode to be in the same atmosphere as the cells; in this closed system, the cells could be subjected to a high concentration of oxygen, and could be viewed through the glass top of the chamber. The volume of the incubating medium was 300–400 μl. A drawing of the chamber is shown in Fig. 1.

Recording

The membrane potentials were recorded with micro-electrodes of tip diameter less than 1 μ, and resistances between 5 and 15 MΩ. These were attached to a non-polarizable electrode, connected to a Bak amplifier, and a Tektronix 502 oscilloscope. The electrode was mounted on a de Fonbrune micromanipulator, and the cells viewed through a Zeiss stereo-microscope II, 1958 model. They were usually penetrated under a magnification of 100 times.

Media

For more than a year attempts were made to record membrane potentials at 23 and 37° C in the Krebs–Ringer bicarbonate–glucose saline, of the same composition as had been used for studying guinea-pig cerebral slices *in vitro* (Li & McIlwain, 1957; Hillman & McIlwain, 1961). However, in this medium only 20–30 mV potentials could be recorded from the cells (Table 2, column 2).

Therefore many variations in the K^+, Na^+, buffer, and glucose were tried. A solution containing the following constituents as final concentrations (mM) was finally adopted, because at room temperature it permitted mean membrane potentials of 39 mV, which could be significantly and reversibly altered (Table 1): NaCl 84, KCl 5, KH_2PO_4 1·24, $MgSO_4$ 1·3, $CaCl_2$ 2·8, $NaHCO_3$ 26 and glucose 50, equilibrated for at least 10 min with 95 %

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O_2, 5 % CO_2, and at pH 7·4. Throughout the paper this solution will be described as 'the Krebs–Ringer'. The only difference between this and the solution used for cerebral slices *in vitro* (Li & McIlwain, 1957) was that the glucose concentration was raised to 50 mM, and the NaCl lowered to 84 mM, leaving the same final tonicity.

In some of the experiments on hypoxia, thirty-five cells were placed on the glass in sucrose, the oxygenated Krebs–Ringer bicarbonate glucose saline was pipetted on to them, and they were gassed by passing the 95 % O_2:5 % CO_2 mixture into the chamber for 1 min; the outlet of the chamber was then closed. After a further minute, ten of the cells were penetrated, and the resting potentials were measured. The chamber was then flushed out with 95 % N_2:5 % CO_2 mixture for a further minute, it was closed, and a further ten cells penetrated; the gas phase was then changed back to the 95 % O_2:5 % CO_2 mixture, and the

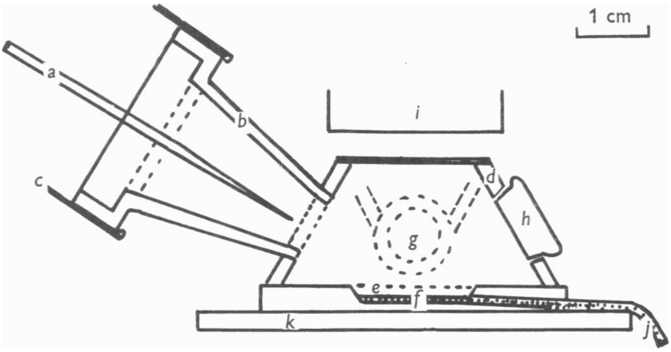

Fig. 1. Diagram of cross-section of chamber for incubating isolated neurones of the rabbit Deiters nucleus *in vitro*, at 22° C, and penetrating them with an intracellular micro-electrode under microscopic vision; *a* is the glass micro-electrode controlled by a de Fonbrune micromanipulator; *b* is a plastic cone; *c* is a latex tube to keep it air tight; *d* is the conical chamber standing on a plastic disk *k* which fits the microscope stage; *e* is a depression containing the incubating medium, *f*, connected through *j* to earth; *g* is the inlet of a cone (not shown) through which the gases are admitted, and another electrode may be introduced; *h* is the plastic stopper closing the hole through which the cells on a piece of glass are placed in the incubating fluid under microscopic vision, *i*. The chamber is fixed between the microscope objective and stage. The scale is given by the horizontal bar, 1 cm.

last ten cells penetrated. These latter cells had thus been subjected to all three different gas phases. In further experiments on hypoxia the cells were placed *from the beginning* in the Krebs–Ringer medium gassed with 95 % N_2:5 % CO_2, and also subjected to this same gas mixture; this produced much more drastic changes (see Results).

Where incubation in different glucose concentrations was studied (Table 2), the tonicity was adjusted by altering the Na^+ concentration. Furthermore, in these experiments and in the Na^+ experiments, groups of twelve cells were put in 0·25 M sucrose on separate pieces of glass, and the Krebs–Ringer used was pipetted on to each piece of glass 2–3 min before beginning penetration of the cells. The other groups were covered by small closed inverted funnels containing a 95 % O_2:5 % CO_2 atmosphere at room temperature for a period up to about 20 min before sucrose was replaced by the regular medium, and their membrane potential examined.

Sodium chloride was substituted for KCl, and Na_2HPO_4 for KH_2PO_4 in the low K^+ experiments, and choline chloride (Merck) was used to replace NaCl in the experiments on the effects of Na^+. In the Na^+-deficient medium, the $NaHCO_3$ was replaced by tris–HCl buffer. The final Na^+ concentrations tested were 0, 26, 84, 110 and 150 mM. The latter had to be 40 mM hypertonic to accommodate the 50 mM glucose which was necessary (Table 2). The ganglioside preparation of human embryo brain was kindly provided by Docent L. Svennerholm, and prepared by the method of Svennerholm (1956).

Procedure

The rabbits were anaesthetized with ether, their carotid arteries were severed, and they were exsanguinated, as described elsewhere (Hillman and Hydén (1965). Freehand, 35–70 neurones with their surrounding neuroglia were dissected out from the Deiters nucleus with a 20μ stainless-steel wire in 0·25 M sucrose with slight staining with methylene blue, as previously described (Hydén, 1959). In pilot experiments, in which cells were dissected out *without* this staining, no significant differences for values of membrane potential were found using the two techniques, but, as the latter took much longer, the former was adopted as routine. The neurones were separated freehand by micro-manipulation of the steel wire. It was found that, if they were placed on glass slides approximately 5×5 mm at room temperature, the cells stuck well; the glass slides had to be scrupulously clean, having been washed in ether and then absolute ethanol, and handled with forceps. The sucrose solution was then drawn off with a micropipette, and it was replaced by the Krebs–Ringer bicarbonate–glucose saline to cover the cells to a depth of 50–100μ.

In *every* experiment the resting potentials were recorded from 20–60 neurones, prepared from the same animal in the same solutions, and penetrated under direct vision. Whenever the chamber had to be opened to change the incubating medium, or adjust the position of the cells, 95 % O_2:5 % CO_2 gas mixture was passed over them for 60 sec, before recording was continued. Resting potentials could be recorded within a minute of changing the medium.

The resting potentials were observed on an oscilloscope during penetration of a cell under direct vision. These potentials could sometimes be maintained for some minutes, but this was not usually done. A second penetration of the same cell often gave almost as high a potential, which disappeared on repeated penetrations.

In the experiments reported in this paper, the artifacts of moving the electrodes along the surface of the glass, pressing them, or agitating them were less than 2 mV, and the accuracy of reading was 2 mV. The same electrode was used throughout the experiment. Tip potentials of up to 5 mV were backed off, and electrodes with greater tip potentials were discarded (Adrian, 1956).

It was found that seventy cells were the maximum which could be taken out in one session. Up to ten of these were lost during preparation and this permitted six conditions to be tested on each of ten cells from the same animal in the same experiment.

RESULTS

Resting membrane potential

The shapes of the cells, their nuclei and the shortness of the cut ends of the dendrites can be seen (Fig. 2). When the cells were taken out in sucrose they appeared slightly blue owing to the methylene blue used to show them up. This colour was lost within 2–3 min after they were placed in the modified Krebs–Ringer solution used here. At 23° C, isolated neurones of Dieters' nucleus of rabbit in an oxygenated atmosphere, in the modified Krebs–Ringer bicarbonate solution (50 mM glucose), gave resting potentials of $39 \pm 4{\cdot}3$ mV ($n = 120$) (Table 1*a*) No incubation period was needed before recording was made. The aim of the present experiments was to examine the values of the resting potentials and no special effort was made to record them for longer than several minutes. The results are presented in Table 1 *a*.

Voltages higher than 20 mV were never recorded on penetration of neuroglia, and their study could not be pursued. Their cytoplasm is

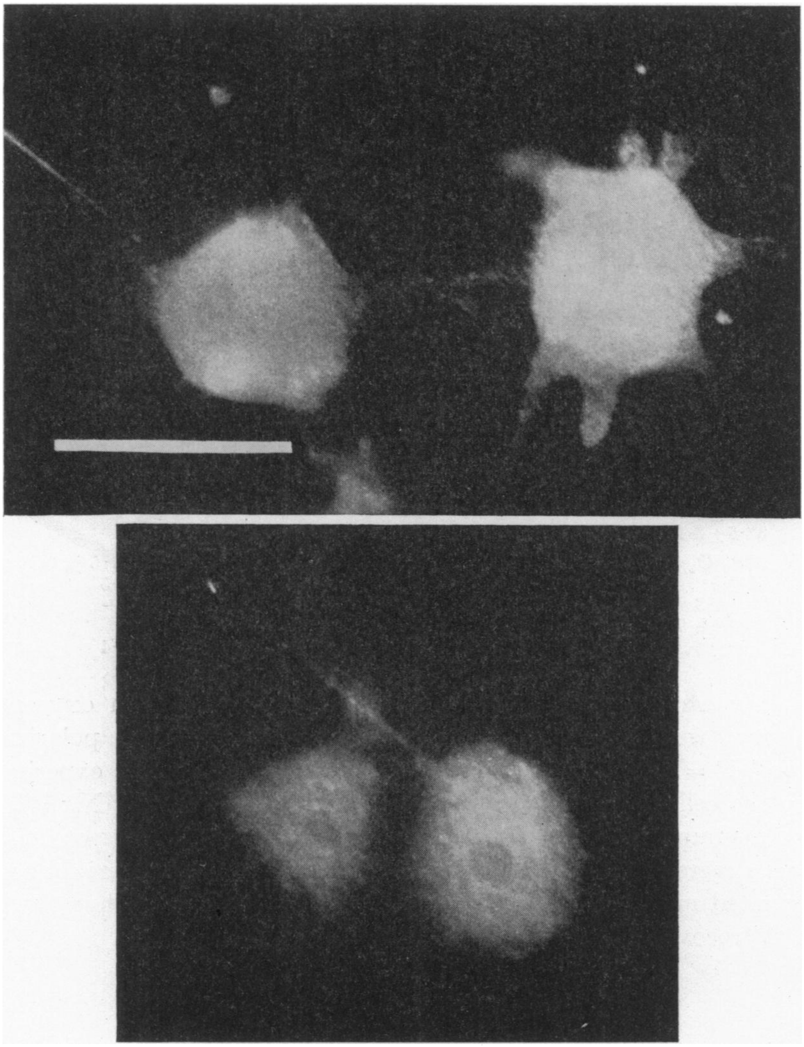

Fig. 2. Micro-electrodes penetrating isolated neurones of the Deiters nucleus at 23° C. The medium is the modified Krebs–Ringer bicarbonate–glucose saline described in the Methods section. Note the shape of the cells; their size is given by the horizontal bar, which is 50 μ. In some cells the nuclei are visible.

reported to be in filamentous tubes of membrane, which would render penetration difficult (Mugnaido & Wallberg, 1964).

Hypoxia

The resting potential of cells could be significantly decreased (each experiment $P < 0{\cdot}001$) from a mean of $41 \pm 6{\cdot}4$ to a mean of $25 \pm 6{\cdot}2$ mV ($n = 40$) by changing the atmosphere over them from a 95 % O_2:5 % CO_2 to a 95 % N_2:5 % CO_2 mixture and leaving them in this atmosphere for a further minute. Re-introduction of the oxygenated atmosphere always significantly increased (each experiment $P = 0{\cdot}01$) the negative voltages recorded, back to an over-all mean of $35 \pm 4{\cdot}6$ mV ($n = 40$) (Table 1*b*).

TABLE 1 (*a*), (*b*). The trend of membrane potentials of neurones of the Deiters nucleus of rabbit in three groups of ten cells each in the same experiment (*a*) in three periods of 95 % O_2:5 % CO_2 (*b*) in changing from 95 % O_2:5 % CO_2 to 95 % N_2:5 % CO_2 and back to 95 % O_2:5 % CO_2. In all tables and figures in this paper, each number or point represents the mean ± S.D. of ten different cells, and numbers on the same horizontal line, in the tables, or with the same symbols on a particular graph, are from the same experiment

Membrane potential (mV, mean ± S.D.).

	1st 10	2nd 10	3rd 10
(*a*)	95 % O_2:5 % CO_2	95 % O_2:5 % CO_2	95 % O_2:5 % CO_2
	$41{\cdot}5 \pm 6$	$39{\cdot}5 \pm 3$	36 ± 3
	$37 \pm 3{\cdot}5$	40 ± 4	$37 \pm 3{\cdot}5$
	$44{\cdot}5 \pm 7{\cdot}7$	40 ± 4	$39{\cdot}5 \pm 4$
	$38{\cdot}5 \pm 3{\cdot}4$	$39 \pm 4{\cdot}5$	$39 \pm 3{\cdot}1$
(*b*)	95 % O_2:5 % CO_2	95 % N_2:5 % CO_2	95 % O_2:5 % CO_2
	43 ± 5	$25{\cdot}5 \pm 7$	$39{\cdot}5 \pm 5{\cdot}5$
	$36 \pm 7{\cdot}3$	$23{\cdot}5 \pm 7{\cdot}6$	$31 \pm 4{\cdot}5$
	$43{\cdot}3 \pm 8{\cdot}5$	$25{\cdot}5 \pm 4{\cdot}4$	$37{\cdot}5 \pm 5$
	$41{\cdot}5 \pm 5{\cdot}0$	$25{\cdot}0 \pm 5{\cdot}0$	$38{\cdot}5 \pm 4{\cdot}4$

In four earlier experiments when the control values in oxygenated conditions were 30–33 mV, hypoxia also decreased the polarization (over-all $P = 0{\cdot}01$) but 'recovery' did not occur. In four experiments where the cells were placed *from the beginning* in the 95 % N_2:5 % CO_2 gassed medium, with the same gas as atmosphere, the resting potentials of the cells were only 12 ± 3 mV, and even on subsequent incubation in oxygenated medium they were only 15 ± 4 mV ($n = 40$). Thus the cells did not 'recover' from early hypoxia.

Glucose

In the absence of added glucose, but the presumed presence of endogenous substrate, resting potentials were between means of 7·4 and 23 mV. They were larger with 10 mM glucose, and maximal with a mean of 38·5–40·5 mV, with 50 mM glucose (Table 2). The smaller resting potentials in 100 mM glucose may be due to a lowering of the Na^+ concentration that was necessary in order to maintain tonicity (see below). In all cases, the 100 mM glucose concentration made the cells look more opaque, and they came unstuck from the glass more easily on penetration.

TABLE 2. Effect of placing the cells in media containing different glucose concentrations, the tonicity being kept constant by adjustment of the Na^+ in the medium. As in all experiments, the medium was added 2–3 min before cell penetration was commenced

Membrane potential, mV, in different glucose concentrations (mM).			
0	10	50	100
$12 \pm 2{\cdot}9$	$20 \pm 4{\cdot}1$	$40 \pm 4{\cdot}7$	$19{\cdot}5 \pm 4{\cdot}4$
$17 \pm 2{\cdot}5$	$29 \pm 3{\cdot}9$	$40{\cdot}5 \pm 5{\cdot}5$	$32 \pm 4{\cdot}8$
$23 \pm 7{\cdot}0$	$26{\cdot}5 \pm 4{\cdot}8$	$38{\cdot}5 \pm 4{\cdot}8$	$18 \pm 4{\cdot}2$
$7{\cdot}4 \pm 1{\cdot}7$	$20 \pm 3{\cdot}5$	$40 \pm 5{\cdot}7$	$20 \pm 3{\cdot}5$

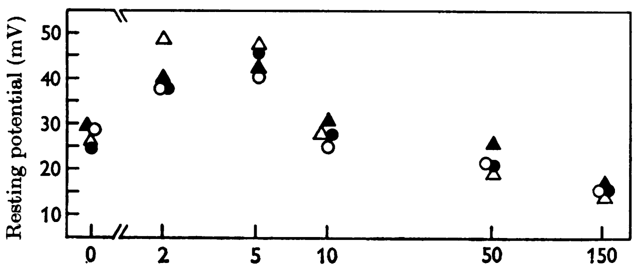

Fig. 3. The effect of changing the potassium concentration of the medium on the mean membrane potential of the rabbit Deiters neurones *in vitro* at 23° C. The same symbol is from the same experiment, and the range of standard deviations for each group of ten cells in a particular K^+ concentration was between $\pm 2{\cdot}4$ and $\pm 5{\cdot}3$ mV. Note that the abscissa is logarithmic.

Potassium

Small transmembrane voltages, means of 25–29 mV, were recorded in the absence of added K^+. However, the electrodes contained 2·7 M-KCl, and some leakage may have occurred. Raising the medium K^+ concentration to 2 mM by washing off the K^+-deficient medium and replacing it by 2 mM-K^+ medium increased the membrane potential to means of between 37 and 49 mV, and further increase to 5 mM increased it further in three out of four experiments. In all cases, the 10, 50 and 150 mM-K^+ medium concentrations progressively lowered the resting potentials to means of 14–17 mV in the latter medium. These effects may have been exaggerated by the fact that the ten cells examined in the final 150 mM-K^+ medium had already been subjected to the 0, 2, 5, 10, 50 mM-K^+ solutions (Fig. 3).

Sodium

The potentials recorded were significantly less in the absence of added Na^+, and in the 26, 84 and 150 mM-Na^+ media than in the 110 mM-Na^+ medium (Fig. 4). The 150 mM-Na^+ medium was also 50 mM in glucose, and was 40 mM hypertonic; all the others were isotonic. In the experiments, the first concentrations tested were always different to avoid a possible effect of time, and the chamber was washed out 2–3 times before the new concentration was introduced. However, the 84 mM-Na^+ always permitted less polarization than the immediately higher and lower Na^+ media tested.

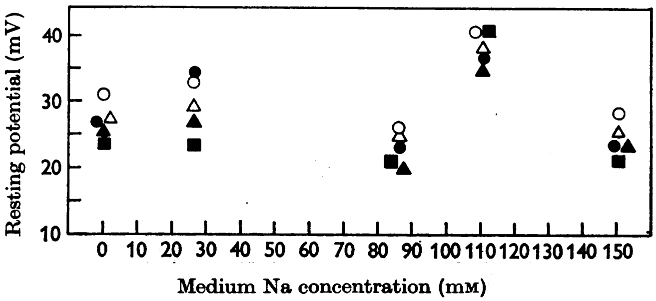

Fig. 4. The effect of different sodium concentrations on the mean membrane potential of the rabbit Deiters neurones *in vitro* at 23° C. The Na concentration was adjusted by substitution of choline chloride. The range of the standard deviations for each group of ten cells in a particular Na^+ concentration was between $\pm 2{\cdot}8$ and $\pm 5{\cdot}3$ mV.

Ganglioside preparation

In four experiments a ganglioside preparation (0·3 mg/ml. of medium) increased the resting potential by an average of 19 %, with the significance for each experiment shown (Table 3).

TABLE 3. Effect of adding 0·3 mg of ganglioside preparation/ml. of modified Krebs–Ringer solution. The *P* value is the significance of the rise between columns 1 and 2

Membrane potential (mV)		
Normal medium	+0·3 mg/ml. ganglioside	*P* value
$49 \pm 12{\cdot}0$	$56 \pm 4{\cdot}5$	0·2
$42{\cdot}5 \pm 5{\cdot}2$	$55{\cdot}5 \pm 4{\cdot}2$	0·001
$44{\cdot}5 \pm 4{\cdot}5$	$52{\cdot}5 \pm 5{\cdot}0$	0·05
$42 \pm 5{\cdot}2$	$50{\cdot}5 \pm 5{\cdot}0$	0·01
$47 \pm 8{\cdot}0$	$54 \pm 5{\cdot}5$	0·05

DISCUSSION

Resting membrane potential

These cells were studied at room temperature (23° C) because, first, at this temperature they had membrane potentials which could be reversibly and significantly changed; secondly, they did not stick to glass at 37° C and so penetration was very difficult; thirdly, a satisfactory system to prevent the medium around them evaporating as they were warmed from 23 to 37° C was not achieved; fourthly, they appeared swollen at 37° C.

It might be considered that a mean of 39 mV (Table 1) was rather low for viable cells. Since reports of mammalian tissues studied at this temperature have not been found, and the temperature coefficient between 23 and 37° C of the membrane potentials is not known, it is difficult to compare these values with others of similar cells. Furthermore, workers examining electrical activity in spinal or cerebral motoneurones (Frank & Fuortes, 1955; Phillips, 1956), for example, chose cells with larger resting potentials to study. Recently, Ito, Hongo, Yoshida, Okada & Obata (1964) have recorded in Deiters' neurones of anaesthetized cats resting potentials of 50–70 mV. In mammalian pyramidal cells *in vivo* (Phillips, 1956; Sawa, Maruyama, Kaji & Hanai, 1960), and in cells which gave spike discharges in cerebral slices *in vitro* (Hillman, Campbell & McIlwain, 1963), the mean resting potentials were generally 50–55 mV, which is only 10–15 mV higher than those found here at the lower temperature. However, addition of a ganglioside preparation increased the resting potential to within the range found *in vivo* (Table 3). Kuffler & Potter (1964) studied the resting membrane potentials of leech neurones and neuroglia at room temperature (22–23° C). They found values for the neurones of 40–50 mV, and of the large neuroglia between 60 and 75 mV.

Hypoxia

Hypoxia caused a considerable and significant decrease in the membrane potentials of these neurones (Table 1*b*), more profound if the tissue was isolated in hypoxic conditions than if the cells had been first enabled to polarize to their normal levels, as was found in cerebral slices *in vitro* (Hillman, 1961). The recovery after re-introduction of oxygen in the present experiments was the first clear evidence that the signals were not artifacts.

Glucose

In the absence of glucose some of the smallest voltages were recorded (Table 2). Owing to the real possibilities of contamination, and the probably varying glucose concentrations in the cells at the time of isolation, it is likely that the least polarization represented the state of the cells in the presence of minimal glucose substrate. In mammals the dependence of the polarization on glucose substrate at 37° C has been demonstrated in cerebral slices *in vitro* (Hillman, 1961), and is in marked contrast to squid axons or lobster stretch receptors, which can go on firing several hours in its absence (Baker, Hodgkin & Shaw, 1962; Grampp & Edström, 1963).

The effect of 100 mM glucose in allowing lesser cell polarization than the 50 mM used otherwise throughout the experiments (Table 2) is probably explained by the fact that the medium had to be lowered in Na^+ to keep it isotonic. It can be seen (Fig. 4) that 84 mM-Na^+ always permitted smaller membrane potentials than the immediately higher or lower Na^+ concentrations. The explanation for the finding that a 50 mM glucose medium was necessary for maximum membrane potential is difficult, as it is five times higher than that of the serum. Adult mammalian tissues *in vitro* have not been studied in the presence of this glucose concentration, but growth of cerebral and retinal tissues in culture has generally required enhanced glucose concentrations (Murray, 1964; Hansson & Sourander, 1964), which is why the higher concentration was tested.

When the dendrites are cut (Fig. 1) the cell would become more leaky to both K^+ and Na^+, and so more energy might have to be expended than in the normal tissue *in vivo* to support the gradients against the medium (Joanny & Hillman, 1963, 1964). It has recently been shown that anaesthesia has the effect of at least doubling the cerebral glucose of mice (Mayman, Gatfield & Breckenridge, 1964), which might indicate a greater glucose 'requirement'. The present cells might have used more glucose during the anaesthesia in which the rabbits were killed, and also during the subsequent isolation.

Potassium

In every experiment the K^+-deficient media permitted lower membrane potentials, but 2–5 mM (the serum and cerebrospinal fluid concentrations) gave the maximum values, as they do in brain slices (Hillman & McIlwain, 1961), and as they give maximum K^+ gradients in the same tissue (Pappius & Elliott, 1956; Pappius, Rosenfeld, Johnson & Elliott, 1958). Successive increase of K^+ concentration gradually depolarized the cells. The slope of this depolarization was 30–35 mV for a tenfold increase in external K^+ concentration (Fig. 3), compared with a value of 58 mV if the cells were acting as K^+ electrodes (Hodgkin, 1951). The possible explanations for this small value, and also the low resting potentials are, firstly, irreversible changes to the cells on isolating them, and, secondly, the temperature being about 15° C lower than that at which the enzyme systems normally operate *in vivo*.

The presence of potentials of 10–15 mV with high K^+ (Fig. 3), in early hypoxia and in the absence of substrate may be due to some residual concentration of K^+ by the cells. Guinea-pig cerebral slices subjected in the absence of added substrate to hypoxia still maintain a K^+ gradient to the medium of at least 2–3, which would probably imply an intracellular/extracellular gradient of twice this size (Joanny & Hillman, 1963, 1964). This could account for the residual membrane potentials in the present cells in these drastic conditions.

A characteristic of these cells has been the ability to record within 1 or 2 min of isolating them. This is in marked contrast to cerebral slices, which required 20–40 min incubation to reconcentrate the K^+, expel the Na^+, resynthesize the phosphocreatine and repolarize the cells (Krebs, Eggleston & Terner, 1951; McIlwain, 1952). The greater distance for diffusion of substrates and metabolites in cerebral slices about 300 μ thick compared with cells about 50 μ diameter is a possible explanation for this difference.

Sodium

The reasons why these concentrations were chosen were that 0 and 26 mM had been studied in cerebral slices (Hillman & McIlwain, 1961), 84 mM was an intermediate concentration, which could be easily made up, 110 mM was the control condition, and 150 mM is the usual Na^+ concentration in serum and cerebrospinal fluid. In 84 mM-Na^+ concentrations in which some Na^+ had been replaced by choline chloride (Fig. 4), the resting potentials were less than in all the other concentrations (including no added Na^+). This contrasted with the cerebral slice *in vitro*, in which the cell polarization and the tissue K^+ were unaffected by lowering the Na^+ from 150 to 80 mM (Hillman & McIlwain, 1961; Joanny & Hillman, 1964). The presence of cell polarization in low Na^+ media contrasted with much less in low K^+ media implied that the neurones of the Deiters nucleus required much less Na^+ to maintain their K^+ gradients than did cellular elements in cerebral slices. A reserve in interpreting the differences between these findings emphasizes the difference between the present system of a single, identified cell and the heterogeneous cerebral slice.

Ganglioside preparation

Many amino acids and other agents applied iontophoretically to cerebral and spinal motoneurones depolarize them (Krnjević & Phillis, 1963; Curtis, Perrin & Watkins, 1960; Curtis, Phillis & Watkins, 1960), but a ganglioside preparation here had the effect of *increasing* cellular polarization, as it did in cerebral slices (Hillman, 1961). The values of their membrane potentials at 23° C (Table 3) then fell within the range found at 37° C in cells identified by antidromic stimulation as pyramidal motoneurones (Phillips, 1956; Li, 1959; Sawa *et al.* 1960). The gangliosides are *n*-acetyl-neuraminic acid containing fatty acids, which have a variety of biochemical properties, including that of restoring to cerebral slices their ability to respond biochemically to electrical stimulation, after it had been lost owing to the application of basic proteins (for review, see McIlwain, 1963).

The neurone of the Deiters nucleus as a model

The significant changes induced by short periods of hypoxia, absence of added substrate, changes in K^+, Na^+ and addition of ganglioside encouraged the possibility of the use of the neurone of the Deiters nucleus as a model. Survey of the literature has failed to reveal previous reports of cell bodies completely isolated from a tissue showing resting potentials. They have, however, been reported in other tissues *in vitro*, for example in the isolated stretch receptor organs of invertebrates (Kuffler, 1954; Grampp & Edström, 1963), cultures of mammalian neurones (Hild & Tasaki, 1962; Crane & Peterson, 1963), and cells in cerebral slices *in vitro* (Li & McIlwain, 1957). There is further evidence for the viability of these cells: (i) microscopically, they appear neither swollen nor shrunken (Fig. 2), and have a rich synaptic covering on their surfaces (Hydén & Pigon, 1960), (ii) they show considerable activity of oxidative enzymes (Hydén & Pigon, 1960; Hamberger, 1961), (iii) they have been shown to respire actively (Hydén and Lange, 1965), (iv) they break down ATP at a mean rate of $2{\cdot}4 \pm 0{\cdot}2\,\mu\mu$moles/cell/hr, and then take up the liberated phosphate, unless inhibited from doing so by an uncoupler of oxidative phosphorylation (Hillman and Hydén, 1965). In order for this system to be used successfully as a model, substances of physiological and pharmacological interest should be tested on it. However, its best justification would be the demonstration of excitability of the cell membrane, and this possibility is being studied.

SUMMARY

1. On penetration of neurones *in vitro* at 23° C, isolated from the rabbit Deiters nuclei, resting potentials of 39 mV $\pm$ 4·3 (n = 120) were recorded in a Krebs–Ringer bicarbonate–glucose saline, containing 50 mM glucose.

2. Membrane potentials decreased from $41 \pm 6{\cdot}4$ to $25 \pm 6{\cdot}2$ during hypoxia, and recovered to $35 \pm 4{\cdot}6$ mV (n = 40) on reintroduction of 95 % O_2:5 % CO_2.

3. Resting potentials between 7·4 and 23 mV were recorded in the absence of added substrate; they were maximal (39·5 $\pm$ 5 mV) with 50 mM glucose; at 10 mM, or 100 mM glucose concentrations, the membrane potentials were smaller.

4. Potassium ions (2–5 mM) allowed the maximum resting potential of

means between 37 and 49 mV, of 0, 2, 5, 10 and 150 mM medium K^+ concentrations tested.

5. Sodium ions (110 mM) allowed the maximum membrane potentials, of means between 40·5 and 46 mV, and 84 mM the minimum, between 25 and 31 mV, of 0, 26, 84, 110 and 150 mM-Na^+ concentrations tested.

6. A ganglioside preparation (0·3 mg/ml. of medium) *increased* the resting potential from means between 42 and 49 mV to means between 50·5 and 56 mV.

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